mutant. Most prominently, the level of phosphorylation of the pol II C-terminal domain (CTD) is significantly decreased both at Ser2 and Ser5, the positions in the CTD repeat usually phosphorylated in elongating pol II (Sims et al., 2004). As CTD phosphorylation is required for the recruitment of several factors required for mRNA maturation, this defect is likely the cause of the other defects detected, including the lack of association of *GAL1* mRNA with polysomes. Taken together, these results suggest that Gal4 ubiquitylation and turnover play a key role in Gal4 activation at a level that affects mRNA maturation. The authors suggest a model in which Gal4 destruction promotes disassembly of the initiation complex, facilitating a transition to a productive elongation complex.

A number of important issues are illuminated by this work and suggest future experiments. A central question that remains to be addressed is whether Gal4c is the only target of Dsg1/Mdm30 that is relevant to *GAL* gene activation. While Muratani et al. (2005) clearly demonstrate effects of Dsg1/Mdm30 on Gal4 ubiquitylation and stability, it remains possible that Dsg1/Mdm30 also modifies and destabilizes a more globally acting transcription, chromatin, or RNA processing factor. A challenging experiment, to map and mutate the Dsg1/Mdm30-dependent Gal4 ubiquitylation sites, would address whether Gal4 ubiquitylation is the whole story with respect to the effects observed.

Assuming that Gal4 is the relevant target, it will be important to determine whether ubiquitylation per se or ubiquitin-dependent proteolysis is required for activation. In an earlier study, direct fusion of ubiquitin to Gal4-VP16 compensated for the loss of normal ubiquitylation of this activator (Salghetti et al., 2001). The resulting fusion protein was competent for activation yet stable, demonstrating that the contributions of protein ubiquitylation and degradation can be uncoupled. Modification of the Gal4 transcription activation domain by Dsg1/Mdm30-mediated ubiquitylation could potentially affect the recruitment of specific factors required for subsequent events. An interesting possibility is that Dsg1/Mdm30 may recruit components of the proteasome, which have been argued to play nonproteolytic roles in transcription elongation (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). If Gal4 turnover is indeed essential for activation, this would suggest that activated Gal4 must be regenerated with each new round of transcription. For the *GAL* genes, then, each round of transcription could be viewed as a "pioneer" round requiring reassembly of an initiation complex.

Given the importance of Gal4 ubiquitylation, the regulation of Dsg1/Mdm30 itself becomes an important issue. Is association of Dsg1/Mdm30 with the *GAL1-GAL10* UAS regulated by galactose or dependent upon known coactivator complexes, such as SAGA? Is Dsg1/Mdm30 itself part of a known coactivator complex? Does it activate Gal4 in conditions where galactose is not needed for *GAL* gene induction, such as in a *grr1Δ* mutant grown in raffinose?

Finally, and most importantly, the mechanism by which activator function is tied to a productive transition from initiation to elongation remains to be elucidated. Future studies will certainly focus on CTD phosphorylation, whose levels are significantly reduced in strains lacking Dsg1/Mdm30 and whose central role in coordinating RNA synthesis and maturation is well established. An investigation of how the ubiquitylation and elimination of a most well-studied activator, Gal4, leads to proper CTD phosphorylation and progression of the transcription cycle will likely provide general insights into the control of gene expression. The work of Muratani et al. (2005), therefore, both adds significantly to our knowledge in the area of transcriptional regulation and sets the stage for what will likely include additional surprising results.

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San1p, Checking up on Nuclear Proteins

Cellular quality control mechanisms perform vital tasks by ensuring that the proteome reflects precisely the information encoded by the genome. In this issue of *Cell*, Gardner et al. (2005) report the discovery of a novel protein quality control system that resides in the nucleus. Central to this system is the E3 ligase San1p, which monitors nuclear proteins and targets aberrant species for destruction.

The life of a protein can be terminated for many reasons. Numerous cellular processes require the timed

breakdown of regulatory factors. Proteins that are not subjected to regulated degradation deteriorate eventually and become detrimental to cellular function. To protect the cell from damage caused by malfunctioning polypeptides, protein quality control (PQC) systems have evolved that target defective proteins and attempt to restore their native conformation. Only proteins beyond rescue are destroyed. These proteins, however, are not the only source of aberrant proteins. Experimental data suggest that approximately 30% of all newly synthesized proteins fail to acquire their native structure (Schubert et al., 2000). To ensure that these waste products of protein synthesis are degraded promptly, PQC systems are located in compartments where protein maturation takes place: the cytosol, the endoplasmic reticulum (ER), and mitochondria (Arnold and Langer, 2002; Cyr et al., 2002; Hirsch et al., 2004). Most likely, these PQC systems also take charge of abnormal proteins that have been damaged posttranslationally by aging or environmental stress. The importance of PQC systems for the maintenance of cellular homeostasis becomes immediately evident from the various pathologies such as Alzheimer's, Huntington's, and Parkinson's diseases, which are all linked to defects in protein quality control (Selkoe, 2003).

The hallmarks of a PQC system are proof-reading, refolding, and degradation. Central to protein quality control is the proof-reading step where proteins are either returned for refolding or sentenced for destruction. One of the factors that judges over the fate of malfolded proteins is the cytosolic U box ubiquitin-ligase CHIP (Cyr et al., 2002). Equipped with a tetratricopeptide repeat domain, CHIP interacts with chaperones such as hsp70-hsp90 and screens their cargo. Folding-incompetent proteins are ubiquitinated by CHIP and subsequently degraded. The stoichiometry between chaperones and CHIP determines the balance between folding and degradation: elevated levels of CHIP increase the stringency of the proof-reading step by enhancing the rate at which degradation occurs.

While our understanding of the mechanisms that govern quality control in the cytosol and the ER advances, evidence for a nuclear PQC counterpart has remained elusive. An astounding number of pathways for the regulated degradation of nuclear substrates have been characterized, but a PQC system that eliminates aberrant proteins from this organelle has not been described so far. Still, such a system must exist since many proteins are restricted to the nucleus by a nuclear localization sequence and thus unavailable for cytosolic PQC systems. This raises the intriguing question as to how dysfunctional proteins are disposed from the nucleus.

In this issue of *Cell*, Gardner et al. (Gardner et al., 2005) come forward with the first answers to this question using a sophisticated approach: certain mutations do not impair the biological activity of a protein but rather attract the attention of a PQC system that inadvertently eliminates the affected protein. The phenotype of such a mutation is caused only by the reduced steady-state levels of the mutated protein. Defects in the PQC system that degrades the mutant protein can therefore restore protein levels and rescue the phenotype. Based on this concept, Gardener et al. searched in silico for a common extragenic suppressor that rescues conditional alleles of nuclear proteins. Such a screen should yield potential components of a nuclear PQC system.

Gardener et al. found two genetic analyses which had previously shown that mutations in the yeast protein San1p rescue the phenotype of temperature-sensitive alleles in two unrelated nuclear proteins. These results made San1p an excellent candidate for a factor that partakes in the degradation of malfolded nuclear proteins. To verify this hypothesis, the authors examined the effect of a *SAN1* deletion on the stability of four distinct nuclear proteins and their mutated counterparts. Indeed, San1p targeted exclusively the malfolded forms of the studied substrates for degradation while leaving the respective native proteins unscathed. It is important to note that lack of *SAN1* does not alter the in vivo function of the native substrates, an observation that lends further support to the conclusion that San1p is part of a quality control and not a regulatory system. But what is the molecular function of San1p in this process? San1p contains a RING finger domain, which is characteristic for a class of ubiquitin ligases that targets proteins for degradation by the proteasome. Using an in vitro assay, the authors confirm that San1p possesses ubiquitin-ligase activity that requires the RING finger domain. In vivo, San1p function depends on its RING finger and nuclear localization. Mislocalization of San1p to the cytosol abrogates the turnover of malfolded nuclear proteins.

Since San1p and CHIP both mediate ubiquitination and thus destruction, it is likely that San1p is a nuclear proof-reading factor that links protein remodeling to the ubiquitin-proteasome pathway. Many questions can now be asked to further our understanding of this exciting new process: Which factors operate upstream of San1p? How is the specificity of this PQC system ensured? The ligase recognizes very different substrates, suggesting that San1p may utilize adaptor proteins to recognize its targets. Could these adaptor proteins be part of a nuclear chaperone system that remodels malfolded proteins? Alternatively, the San1p-dependant PQC system may be much more rigorous than other quality control systems and degrade aberrant proteins without an attempt for repair.

The physiological relevance of San1p is underscored by the fact that deletion of *SAN1* triggers a cellular stress response. Still, lack of San1p does not render yeast cells more susceptible to stress, indicating that back up mechanisms may exist that compensate for loss of San1p. Most certainly, higher eukaryotes possess similar nuclear PQC systems and their importance remains to be established. In contrast to yeast cells, which are probably better equipped to handle cellular stress, defects in nuclear PQC pathways of long-lived cells like neurons may be much more serious. It is quite possible that a number of pathologies will be linked to defects in nuclear protein quality control in the near future.

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